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Na⁺/H⁺ exchange inhibition attenuates hypertrophy and heart failure in 1-wk postinfarction rat myocardium

HIROYUKI YOSHIDA AND MORRIS KARMAZYN

Department of Pharmacology and Toxicology, University
of Western Ontario, London, Ontario, Canada N6A 5C1

Yoshida, Hiroyuki, and Morris Karmazyn. Na⁺/H⁺ exchange inhibition attenuates hypertrophy and heart failure in 1-wk postinfarction rat myocardium. *Am. J. Physiol. Heart Circ. Physiol.* 278: H300–H304, 2000.—Na⁺/H⁺ exchange (NHE) represents a major mechanism for intracellular pH regulation, particularly in the ischemic myocardium. NHE has also been shown to be important in the regulation of cell proliferation and growth. We examined whether inhibition of NHE results in an attenuation of early postinfarction myocyte remodeling responses in the rat. Male Sprague-Dawley rats were randomized to receive either a control diet or an identical diet supplemented with the NHE inhibitor cariporide. After 1 wk, animals were anesthetized, subjected to ligation of the left main coronary artery, and maintained for an additional week, after which time they were anesthetized and intraventricular pressures were obtained. Hearts were removed, and myocytes were isolated to obtain cell dimensions and determine the response to isoproterenol. Body, heart, and lung weights were obtained. Coronary artery ligation in control animals resulted in a significant elevation in left ventricular end-diastolic pressure, as well as increased heart weight- and lung weight-to-body weight ratios, both of which were abrogated by cariporide. Cell length and area significantly increased by 14 and 19.2%, respectively, whereas cell width increased by 4.1% ($P > 0.05$). These cells exhibited a significant hyporesponsiveness to the positive inotropic responses to isoproterenol at the lower drug concentrations (3 and 10 nM). A $<1\%$ dimensional change occurred in myocytes from cariporide-fed animals, and the hyporesponse to isoproterenol was reversed. Cariporide had no effect on infarct size or blood pressure. These studies suggest that the early adaptive hypertrophic response of surviving myocytes is dependent on NHE activity. As such, it is attractive to suggest that NHE inhibition could be an effective therapeutic strategy for prevention of postinfarction remodeling, independent of infarct size or afterload reduction.

sodium-hydrogen exchange; myocyte remodeling; heart failure; cariporide

SODIUM-HYDROGEN EXCHANGE (NHE) represents a major route of proton extrusion in the maintenance of intracellular pH. There is substantial evidence that drugs that inhibit NHE exhibit a marked ability to reduce infarct size (10, 15, 19; reviewed in Refs. 8 and 14). Recently, we demonstrated (13) that oral administration of the NHE-1-specific inhibitor cariporide (HOE 642) reduces mortality and arrhythmias in an acute model of coronary occlusion and reperfusion. There is compelling evidence that postinfarction adaptive and remodeling responses may also involve NHE. For example, stimulation in cardiac protein synthesis can be blocked by NHE inhibitors (12, 23). Orally administered amiloride, a nonspecific NHE inhibitor, reduces fiber diameter in rat coronary ligation (11) and murine-dilated cardiomyopathy models (21). However, the effect of a specific NHE-1 inhibitor on the early postinfarction responses or the properties of surviving myocytes has not been reported. Accordingly, we studied the effect of oral cariporide on left ventricular hemodynamics and on cellular characteristics 1 wk after left coronary artery ligation in the rat.

METHODS

Experimental protocol. We used the well-established coronary artery ligation model to induce myocardial infarction in the rat (9). Male Sprague-Dawley rats (250–350 g) were randomized to receive either a control rat chow or an identical diet containing 3 parts per million cariporide *ad libitum* for 7 days. Animals were then weighed, anesthetized with pentobarbital sodium (50 mg/kg ip), intubated, and artificially ventilated (10 ml/kg, 70 strokes/min) using a rodent respirator (model 683, Harvard Apparatus). Rectal temperature was maintained at 37–38°C. A 6-0 braided silk suture was placed under the left main coronary artery ~2 mm from its origin, and the vessel was ligated; the ligature was put in place but was not tied in sham-operated animals. Negative pressure was then applied, and the chest wall was sutured closed.

Hemodynamic assessment. One week after surgery the animals were weighed and anesthetized, and the left carotid artery was exposed and cannulated with a 3-Fr (1.0-mm OD, Atom Medical Tokyo, Japan) intravenous catheter for blood pressure measurement. The catheter was then descended into the left ventricle (LV) for pressure determination. The heart and lungs were then excised, blotted dry, and weighed.

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Determination of plasma cariporide concentrations. To obtain insight concerning the therapeutic levels of cariporide required to produce salutary effects, blood was collected from five animals in each of the two cariporide-fed groups, and serum samples were assayed for cariporide concentrations using high-pressure liquid chromatography (all assays were performed by the Biochemistry Department, Hoechst Marion Roussel, Frankfurt, Germany).

Measurement of infarct size. After pressure-volume relationships were obtained, the LV was fixed in 10% buffered Formalin (pH 7.4) and infarct size was determined, as previously described, using collagen-specific Picrosirius red staining (20). Briefly, the fixed LV was cut transversely from the apex to base into slices of ~2 mm. These slices were embedded in paraffin, and a thin section (5 µm) was obtained from each slice, mounted on a glass slide, stained with Picrosirius red, and photographed. Photographs were magnified, and epicardial and endocardial circumferences and infarcted-portion length were measured by planimetry. Infarct size was calculated by dividing the sum of the length of the infarcted portion by that of the left ventricular circumference.

Myocyte isolation. One week after coronary ligation myocytes were isolated as described in detail previously (5). Briefly, hearts were removed and perfused for 5 min with Ca²⁺-free HEPES-buffered solution followed by 12 min of perfusion with a solution containing 2 mg/ml collagenase (type I, Sigma, St. Louis, MO) and 0.1 mg/ml protease (type XIV, Sigma). The collagenase was washed out for 2 min with a solution containing 0.2 mM CaCl₂, and then the heart was removed and separated into left and right ventricles. Left ventricular tissues were chopped with scissors and incubated in a solution containing (in mM) 0.2 CaCl₂ and 25 KCl at 37° for 15 min and then filtered and centrifuged for 45 s. Cells were resuspended in 50 ml of solution containing 0.5 mM CaCl₂ for 10 min and gently centrifuged, and the solution was aspirated off. The resultant cells were suspended in a solution containing 1 mM CaCl₂ and diluted to ~100,000 cells/ml. The percentage of rod-shaped cells was determined for each isolation and averaged ~80% irrespective of treatment. There were no differences in yield between any of the treatment groups.

A cell aliquot was transferred to a stage (37°C) of a Zeiss Axiovert 35 inverted microscope equipped with a perfusion chamber and superfused at 1 ml/min with HEPES solution containing 1.8 mM CaCl₂. Resting cell dimensions were measured using a Hamamatsu Argus 10 image processor, after which cells were electrically field-stimulated at 0.5 Hz for 30 min before isoproterenol was added for a 7-min period. Cell

shortening was determined as a percentage of diastolic cell length using a motion analyzer (Colorado Video, Boulder, CO).

Data analysis. Data were analyzed using a multifactorial ANOVA followed by a Student-Newman-Keuls test to locate differences between treatment groups. Differences were considered significant at $P < 0.05$.

RESULTS

Body weight, tissue weight, and infarct size. As summarized in Table 1, no changes in body weights were observed, and the net weight gain in body weights was identical in all groups. Significant increases in heart and lung weights as well as in body weight ratios were identified in control-diet rats subjected to coronary ligation but not in the cariporide group. Infarct size was unaffected by cariporide treatment.

Hemodynamic function. Heart rates and blood pressures were identical in all groups (Table 2). Coronary ligation increased left ventricular end-diastolic pressure (LVEDP) in control hearts by 90% ($P < 0.05$) and by 35% ($P > 0.05$) with cariporide. Positive and negative first derivatives of pressure ($\pm dP/dt$) were significantly reduced by 18 and 34%, respectively, in control-infarcted animals; the former, but not the latter, was significantly blunted by cariporide.

Table 2 also summarizes left ventricular volumes at selected pressures obtained from pressure-volume curves. Although the curve is not shown, a significant rightward shift was obtained in hearts subjected to coronary occlusion such that volumes were significantly higher ($P < 0.05$) at both low and high filling pressures (Table 2) irrespective of cariporide treatment.

Plasma cariporide values. Serum cariporide levels averaged 351 ± 78 and 336 ± 67 ng/ml for the sham and coronary-ligated groups, respectively.

Cell dimensions and β -adrenergic responsiveness. A significant increase in both cell lengths and area were observed in left ventricular myocytes from control animals subjected to coronary occlusion, although cell width was not significantly affected (Fig. 1). These effects were prevented by cariporide.

In terms of responsiveness to isoproterenol (Fig. 2), no significant differences were observed with 3 nM of the agent. Addition of 10 nM isoproterenol revealed a

Table 1. Body, heart, and lung weight and infarct size data

	n	BW1	BW2	Δ BW	HW	HW/BW	LW	LW/BW	IS
Control diet									
Sham	8	260 \pm 7.8	286 \pm 7.5	26.1 \pm 4.7	901 \pm 12.5	3.25 \pm 0.07	1,099 \pm 40.9	3.86 \pm 0.16	
Ligated	8	260 \pm 6.5	281 \pm 7.5	20.7 \pm 3.8	992 \pm 26.7*	3.63 \pm 0.11†	1,320 \pm 86.3*	4.77 \pm 0.42*	30.4 \pm 3.1
					(10%)	(11.7%)	(20.1%)	(23.8%)	
Cariporide diet									
Sham	8	261 \pm 4.4	290 \pm 4.6	29.4 \pm 4.5	919 \pm 25.7	3.17 \pm 0.07	1,062 \pm 32.0	3.7 \pm 0.09	
Ligated	10	263 \pm 5.0	289 \pm 5.0	26.2 \pm 2.4	944 \pm 24.7	3.26 \pm 0.05	1,166 \pm 55.1	4.0 \pm 0.18	34.7 \pm 3.5
					(2.7%)	(2.8%)	(9.8%)	(8.1%)	

Values are means \pm SE measured in grams for body weights (BW) and milligrams for organ weights; n is no. of animals/group. For infarct size (IS) data n = 5 and values are percentage of left ventricle. BW1 and BW2 indicate animal weights at initiation and 7 days after dietary treatment, respectively. HW, heart weight; LW, lung weight. Percentages in parentheses indicate mean change from sham. * $P < 0.05$, † $P < 0.01$ vs. respective sham value.

Table 2. Hemodynamic data from animals 1 wk after coronary artery ligation

	Control Diet			Cariporide Diet		
	Sham	Ligated	%Δ	Sham	Ligated	%Δ
n	8	8		8	10	
Heart rate, beats/min	413 ± 11.3	391 ± 11.3	-5.4	428 ± 7.2	403 ± 7.1	-5.9
SBP, mmHg	128 ± 5.7	113 ± 7.2	-11.2	129 ± 3.5	122 ± 4.2	-5.5
DBP, mmHg	99 ± 4.3	87 ± 5.6	-12.2	102 ± 3.5	95 ± 2.9	-6.9
LVEDP, mmHg	5.8 ± 0.4	11.1 ± 1.3*	91.4	5.1 ± 0.5	6.9 ± 0.99†	35.3
+dP/dt, mmHg/s	9,218 ± 468	7,545 ± 329*	-18.2	9,255 ± 378	8,231 ± 367	-11.1
-dP/dt, mmHg/s	9,629 ± 441	6,353 ± 291*	-34.1	9,827 ± 393	7,388 ± 502*	-24.9

Values are means ± SE; n is no. of animals/group. Mean percentage change (%Δ) between sham and ligated group is also indicated. SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEDP, left ventricular end-diastolic pressure; + and -dP/dt, rates of left ventricular pressure development and relaxation, respectively. **P* < 0.05 vs. respective sham value; †*P* < 0.05 vs. control diet ligated group.

significantly reduced response in myocytes from infarcted hearts, which was prevented by cariporide and overcome by the addition of 30 nM isoproterenol.

DISCUSSION

NHE inhibition is known to reduce infarct size (10, 15, 19) and protect the acutely ischemic and reperfused myocardium in terms of numerous parameters (reviewed in Refs. 8 and 14). Recently, various compelling lines of evidence also implicate the antiporter in postinfarction adaptive hypertrophic responses. For example, stretch-induced hypertrophy in neonatal cardiac myocytes (23) and stretch-induced alkalization in feline papillary muscles can be blocked by NHE inhibitors (3) as can norepinephrine-induced protein synthesis in

cultured rat cardiomyocytes (12). Orally administered amiloride, a potassium-sparing diuretic that is also a nonspecific NHE inhibitor, reduces fiber diameter in rat coronary ligation (11) and murine dilated cardiomyopathy models (21). In addition to these findings, it is interesting that cardiac hypertrophy in the diabetic rat is associated with increased NHE activity in the cardiac myocytes (6). Moreover, the ability of the angiotensin-converting enzyme inhibitor enalapril to reverse this mode of hypertrophy was associated with a normalization of NHE activity (6). Although extrapolation to clinical cardiac hypertrophy must be done cautiously, it

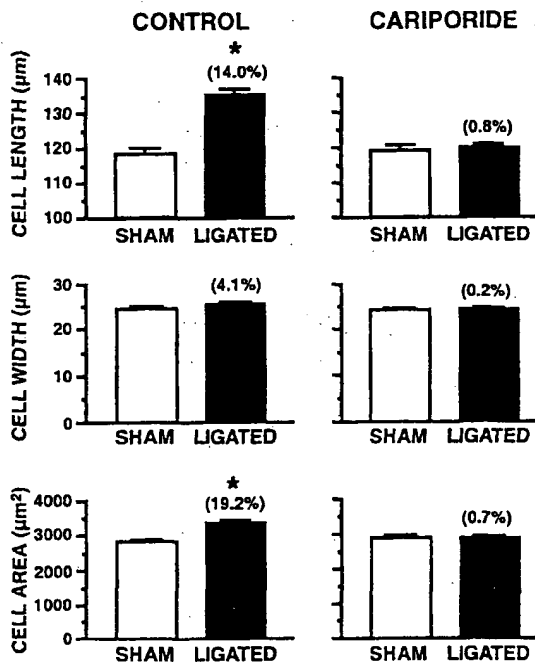


Fig. 1. Myocyte characteristics. Effect of cariporide on dimensions of left ventricular myocytes from sham-operated animals and animals subjected to coronary ligation. Percentage increase from respective sham control is shown in parentheses. Values are means ± SE; n = 10 experiments for all data. *Significantly different (*P* < 0.05) from all other groups.

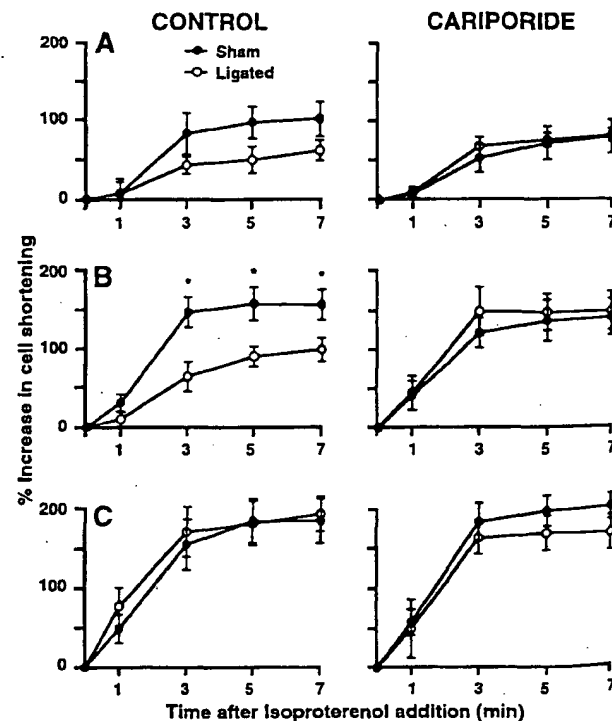


Fig. 2. Effect of cariporide on time-dependent response of isolated cells after addition of different concentrations of isoproterenol (3, 10, and 30 nM; A, B, and C, respectively). Five consecutive shortenings were averaged to obtain each value, and 5 cells for each experiment were averaged and considered as n = 1. Values are means ± SE; n = 10 experiments for all data. *Significantly different (*P* < 0.05) vs. coronary ligated animals.

is nonetheless interesting that left ventricular hypertrophy in hypertensive patients is associated with increased erythrocyte NHE activity, which was significantly correlated with echocardiographic left ventricular mass index (17).

Together, the above findings suggest a potential key role for NHE in mediating ventricular remodeling in the postinfarcted myocardium. Accordingly, we studied the effects of the NHE-1-specific inhibitor cariporide (19) on this process with particular emphasis on surviving left ventricular myocytes that demonstrated increased cell length within days after coronary occlusion (2). We show that the early postinfarction adaptive responses can be attenuated by cariporide. In terms of hemodynamics, cariporide particularly attenuated the increase in LVEDP with less effect on positive or negative dp/dt values, although a significant effect on the former was seen. Cardiac hypertrophy was markedly attenuated by cariporide, and the treatment completely abrogated the increased length of surviving myocytes, further suggesting an important role of the antiporter in cardiac hypertrophic responses. It is important to emphasize that the abrogation of cell remodeling occurred in the absence of infarct size reduction, suggesting that the effect of cariporide involves a direct inhibition of the remodeling process. The failure to affect infarct size was not surprising, despite the well-known infarct-reducing property of NHE inhibition (10, 15, 19), because the animals were subjected to sustained occlusion without reperfusion, thus precluding any potential myocardial salvaging effect of cariporide.

We also examined the response of isolated myocytes to isoproterenol because defective β -adrenergic responses are known to occur in the failing myocardium, although whether this contributes to the heart failure process per se is not completely known (18). The ability of cariporide to reverse the β -adrenergic resistance in surviving myocytes, at least with respect to the 10 nM isoproterenol concentration, was surprising because β -adrenergic desensitization is generally attributed to defects in cell signaling processes, such as the upregulation of β -adrenergic receptor kinase (1, 18). It may be that this salutary effect occurs secondarily to the abrogation of other events in the remodeling process that are dependent on NHE activity. The complexity of the β -adrenergic signaling pathway precludes a firm mechanistic explanation at this time for the salutary effect of NHE inhibition on isoproterenol responses. Indeed, the cellular mechanisms underlying NHE-dependent remodeling or hypertrophy need to be thoroughly investigated, although modulation of intracellular pH or Ca^{2+} is an attractive hypothesis because these are known to be affected by NHE inhibition (reviewed in Refs. 8 and 14).

The precise relative role that NHE plays in the complex hypertrophic and remodeling process is unknown because the latter is governed by numerous paracrine/autocrine factors. However, many of these factors, including endothelin-1 (7), ANG II (16), and α_1 -adrenergic agonists (22), are also potent NHE activa-

tors. As suggested recently (4), NHE may therefore serve as an important downstream regulator contributing to remodeling in response to various hypertrophic factors. If the concept of NHE involvement is valid, the possibility exists for developing novel effective strategies to attenuate postinfarction remodeling responses and the development of heart failure.

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Address for reprint requests and other correspondence: M. Karmazyn, Dept. of Pharmacology and Toxicology, Univ. of Western Ontario, Medical Sciences Bldg., London, Ontario, Canada N6A 5C1 (E-mail: mkarm@julian.uwo.ca).

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REFERENCES

- Bohm, M., M. Flesch, and P. Schnabel. Beta-adrenergic signal transduction in the failing and hypertrophied myocardium. *J. Mol. Med.* 75: 842–848, 1997.
- Capasso, J. M., P. Li, and P. Anversa. Cytosolic calcium transients in myocytes isolated from rats with ischemic heart failure. *Am. J. Physiol. Heart Circ. Physiol.* 265: H1953–H1964, 1993.
- Cingolani, H. E., B. V. Alvarez, I. L. Ennis, and M. C. Camillón de Hurtado. Stretch-induced alkalinization of feline papillary muscle. An autocrine-paracrine system. *Circ. Res.* 83: 775–780, 1998.
- Dostal, D. E., and K. M. Baker. Angiotensin and endothelin: messengers that couple ventricular stretch to the Na^+/H^+ exchanger and cardiac hypertrophy. *Circ. Res.* 83: 870–873, 1998.
- Ebihara, Y., and M. Karmazyn. Inhibition of beta- but not alpha 1-adrenergic responses in isolated hearts and ventricular myocytes by nitric oxide and 8-bromo cyclic GMP. *Cardiovasc. Res.* 32: 622–629, 1996.
- Ennis, I. L., B. V. Alvarez, M. C. Camillón de Hurtado, and H. E. Cingolani. Enalapril induces regression of cardiac hypertrophy and normalization of pH_i regulatory mechanisms. *Hypertension* 31: 961–967, 1998.
- Endoh, M., S. Fujita, H. T. Yang, M. A. Talukder, J. Maruya, and I. Norota. Endothelins: receptor subtypes, signal transduction, regulation of Ca^{2+} transients and contractility in rabbit ventricular myocardium. *Life Sci.* 62: 1485–1489, 1998.
- Fröhlich, O., and M. Karmazyn. The Na-H exchanger revisited: an update on Na-H exchange regulation and the role of the exchanger in hypertension and cardiac function in health and disease. *Cardiovasc. Res.* 36: 138–148, 1997.
- Goldman, S., and T. E. Raya. Rat infarct model of myocardial infarction. *J. Card. Fail.* 1: 169–177, 1995.
- Gumina, R. J., T. Mizumura, N. Beier, P. Schelling, J. J. Schultz, and G. J. Gross. A new sodium/hydrogen exchange inhibitor, EMD 85131, limits infarct size in dogs when administered before or after coronary artery occlusion. *J. Pharmacol. Exp. Ther.* 286: 175–183, 1998.
- Hasegawa, S., M. Nakano, Y. Taniguchi, S. Imai, K. Murata, and T. Suzuki. Effects of Na^+-H^+ exchange blocker amiloride on left ventricular remodeling after anterior myocardial infarction in rats. *Cardiovasc. Drugs Ther.* 9: 823–826, 1995.
- Hori, M., N. Nakatsubo, T. Kagiya, K. Iwai, H. Sato, K. Iwakura, A. Kitabatake, and T. Kamada. The role of Na^+/H^+ exchange in norepinephrine-induced protein synthesis in neonatal cultured cardiomyocytes. *Jpn. Circ. J.* 54: 535–539, 1990.
- Humphreys, R. A., J. V. Haist, S. Chakrabarti, Q. Feng, J. M. O. Arnold, and M. Karmazyn. Orally administered NHE-1 inhibitor cariporide reduces acute responses to coronary occlusion and reperfusion. *Am. J. Physiol. Heart Circ. Physiol.* 276: H749–H757, 1999.
- Karmazyn, M., X. T. Gan, R. A. Humphreys, H. Yoshida, and K. Kusumoto. The myocardial Na^+-H^+ exchange. Structure, regulation and its role in heart disease. *Circ. Res.* 85: 777–786, 1999.

15. Linz, W., A. Albus, P. Crause, W. Jung, A. Weichert, B. A. Schölkens, and W. Scholz. Dose-dependent reduction of myocardial infarct mass in rabbits by the NHE-1 inhibitor cariporide. *Clin. Exp. Hypertens.* 20: 733-749, 1988.
16. Matsui, H., W. H. Barry, C. Livsey, and K. W. Spitzer. Angiotensin II stimulates sodium-hydrogen exchange in adult rabbit ventricular myocytes. *Cardiovasc. Res.* 29: 215-221, 1995.
17. Navarro-Lopez, F., A. Coca, J. C. Pare, A. De La Sierra, X. Bosch, and A. Urbano Marquez. Left ventricular hypertrophy in asymptomatic essential hypertension: its relationship with aldosterone and the increase in sodium-proton exchanger activity. *Eur. Heart. J.* 14, Suppl. J: 38-41, 1993.
18. Post, S. R., H. K. Hammond, and P. A. Insel. Beta-adrenergic receptors and receptor signaling in heart failure. *Annu. Rev. Pharmacol. Toxicol.* 39: 343-360, 1999.
19. Scholz, W., U. Albus, L. Counillon, H. Gögelein, H.-J. Lang, W. Linz, A. Weichert, and B. A. Schölkens. Protective effect of HOE 642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovasc. Res.* 29: 260-268, 1995.
20. Silvestre, J.-S., C. Heymes, A. Oubenaissa, V. Robert, B. Aupetit-Faisant, A. Carayan, B. Swynghewauw, and C. Delcayre. Activation of cardiac aldosterone production in rat myocardial infarction. Effects of angiotensin II receptor blockade and role in cardiac fibrosis. *Circulation* 99: 2694-2701, 1999.
21. Taniguchi, Y., M. Nakano, S. Hasegawa, T. Kanda, S. Imai, T. Suzuki, I. Kobayashi, and R. Nagai. Beneficial effect of amiloride, a $\text{Na}^+\text{-H}^+$ exchange blocker, in a murine model of dilated cardiomyopathy. *Res. Commun. Chem. Pathol. Pharmacol.* 92: 201-210, 1996.
22. Wallert, M. A., and O. Fröhlich. α_1 -Adrenergic stimulation of the Na-H exchange in cardiac myocytes. *Am. J. Physiol. Cell Physiol.* 263: C1096-C1102, 1992.
23. Yamazaki, T., I. Komuro, S. Kudoh, Y. Zou, R. Nagai, R. Aikawa, H. Uozumi, and Y. Yazaki. Role of ion channels and exchanger in mechanical stretch-induced cardiomyocyte hypertrophy. *Circ. Res.* 82: 430-437, 1998.



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